STEM CELLS: LESSONS FROM THE PAST, LESSONS FOR THE FUTURE

IRVING L. WEISSMAN

The beginning of the search for stem cells was the bombing of civilian populations in Hiroshima and Nagasaki, the first use of nuclear weapons in which humans were the target. In retrospect those people who did not die from the blast or the fire but died with the lowest dose of lethal radiation, and the longest time from radiation to death, were those that had destruction of sufficient amounts of their blood forming (hematopoietic) systems that they could not regenerate enough white cells to fend off otherwise non-pathogenic (disease causing) infections, or did not have enough platelets to clot their blood. Higher doses of irradiation killed not only the blood forming system but the stem and progenitor cells of the intestinal tract; because the time of regeneration of the entire intestinal tract is five to seven days, by five to seven days the lining between the body and the intestinal lumen in these higher dose irradiated humans was essentially gone. It was later demonstrated that mice could be given doses of whole body X-irradiation and the same radiation syndromes developed; at the minimal lethal dose, about 8.5 to 10.5 Gy, the mice died at about two weeks post radiation of hematopoietic failure (Ref 1). Shielding even a single bone or the spleen from radiation prevents this irradiation syndrome. Soon thereafter, using inbred strains of mice, whole body radiated mice could be recovered by injection of suspensions of cells from the blood forming organs, for example, the bone marrow (Ref 2). In 1956 three laboratories demonstrated that the injected bone marrow cells regenerated the blood forming system, rather than release radiation factors that caused endogenous cells to repair irradiation damage (Ref 3-5). Then, and now, the only treatment for hematopoietic failure following whole body radiation is transplantation of bone marrow cells, or in fact, the hematopoietic stem cells (HSC) that are responsible entirely for rapid and sustained regeneration of the blood forming system in these hosts (for reviews, see 6,7).
The hematopoietic system is not only destroyed by the minimal doses of lethal X-ray radiation or nuclear radiation, but also by chemotherapeutic agents which, like radiation, largely kill dividing cells. By the 1960s physicians pushing to treat cancer that had spread (metastasized) beyond the primary cancer site to lymphatics and draining lymph nodes, or via the bloodstream to the rest of the body were attempting to take advantage of the fact that cancer cells, are cells that have a high fraction of their cells undergoing cell division. They began using agents that kill dividing cells derived from nitrogen mustards and other empirically discovered agents, as well as broad fields of radiation, to attempt to kill back cancer cells from the approximately $10^{11} - 2 \times 10^{12}$ cancer cells that exist at the time of diagnosis to no cancer cells at all. The field of radiation therapy of local and draining fields of cancer was advanced by the newly developed linear accelerators that deposited most of the radiation at the depth of the tumor rather than at the skin. But this required the development of a quantitative assessment of damage to cancer cells compared to damage to normal cells. To test whether the appearance of dose dependent killing of cancer cells was different in kind to the dose dependent killing of normal cells J. Till and E. McCulloch in Toronto began a quantitative assessment of the radiation sensitivity of a normal cell type as it exists in the body – bone marrow cells useful in transplantation. They began with lowering the dose of bone marrow cells to save just lethally irradiated mice; they found that at sub-radioprotective doses, mice dying at 10 to 15 days developed in their spleens colonies of myeloid and erythroid cells, and these colonies showed a direct variation in their number with the number of bone marrow cells injected (approximately one colony per 7,000 bone marrow cells injected) (Ref 8). To test whether these colonies of myeloerythroid cells derive from single clonogenic precursors, Wu, Becker, Till, Siminovitch and McCulloch preirradiated donors with doses of irradiation that would induce unique chromosome breaks in most hematopoietic cells; surviving cells that had sustained radiation induced and repaired chromosomal breaks which marked for each clonogenic hematopoietic cell (Ref 9). They found that all of the dividing cells within a single mixed cell type spleen colony contained the same unique chromosomal marker, different from all the dividing cells in a second colony, all of which shared their own unique chromosomal marker (Ref 9). They could take the cells within a single spleen colony and reinject them to secondary hosts, often seeing donor derived spleen colonies that had been regenerated from the single cell that generated the first colony; and rarely these cells contained sufficient numbers of regenerative cells that they could radioprotect.
secondary hosts, and in addition, give rise to lymphocytes as well as the myeloerythroid cells within a colony, all bearing markers of the donor injected cells (Ref 10,11). These genetic marking experiments established the fact that there must exist in bone marrow single cells that can both self-renew and generate all of the populations of cells in the blood; these cells were called at that time pluripotent hematopoietic stem cells, a term which has later been modified to multipotent hematopoietic stem cells (HSCs) (Ref 12,13). But knowing that stem cells exist by retrospective analysis of randomly gene marked cells is not the same as having pure populations of HSC for study that can be prospectively isolated.

The Isolation of HSC in Mouse and Man

To search for those cells in bone marrow that contain the activity of HSCs we developed assays for the clonal precursors of T lymphocytes (Ref 14) and of B lymphocytes (Ref 15-16) in addition to the clonal precursors of myeloerythroid cells found in spleen colonies (Ref 8-11). At the point we began, it was already known that the spleen colony-forming cells that Till and McCulloch studied were in fact probably the outcome of oligopotent myeloerythroid progenitors rather than stem cells, and that they were different from colonies that arise at about 14 days – close to the time of death – which come from more primitive hematopoietic cells (Ref 17). For each of those assays a single colony could be derived from $10^3$ to $10^4$ cells, so we had a quantitative assessment for the enrichment of these clonal precursors of T, B and myeloerythroid cells. We then needed a way to fractionate prospectively cells from the bone marrow to put into these colony assays. We chose to produce large numbers of monoclonal antibodies (Ref 18) that detected subsets of cells found in bone marrow, and to use high speed fluorescence activated cells sorters (FACS) to purify these cells (Ref 19). In 1986 we reported a high degree of enrichment of multipotent HSC, and in 1988 their full isolation (Ref 13, 16). At that point mouse hematopoietic multipotent marrow cells represented 1 in 2000 cells in the young adult mouse marrow bones, and were 2000 fold enriched for the ability to radioprotect lethally irradiated hosts by donor derived reconstitution of all blood cell types for life (Ref 13). There were no other cells than these cells in bone marrow capable of long term multi-lineage reconstitution (Ref 20), and at the single cell level these were all multipotents, although some reconstituted for weeks, or a few months, and others reconstituted for life (Ref 21-23). We later showed that these mouse multipotent cells in fact could be subdi-
vided into three subsets – long term HSCs (LT-HSC), short term HSC (ST-HSCs), and multipotent progenitors (MPPs), and that these cells existed in a lineage (Ref 24-26). Of these only LT-HSC self-renewed in apparent perpetuity, while ST-HSCs had a programmed self renewal life span of 6 to 8 weeks, and multipotent progenitors, of less than two weeks (Ref 24-26). The search for human HSCs didn't take long. Using similar assays for clonogenic, lymphoid and myeloerythroid progenitors it was possible to isolate candidate human hematopoietic and multipotent stem and progenitor cells, with their own distinctive and similar cell surface markers (Ref 27-29). We demonstrated long term multilineage reconstitutive activity with these, but no other cells in human hematopoietic tissues by using them to reconstitute human fetal bone, human fetal thymus, human fetal liver, and human fetal lymphoid organs implanted into fully immunodeficient SCID-hu mice (Ref 27, 30, 31). Later, it was shown that these cells too could be divided into LT-HSC and shorter-lived multipotent stem and progenitors (Ref 28). All of this work was carried out at a company of which I was the co-founder (Systemix, now, Cellerant Inc), and an account of the founding of the company and the academic issues involved can be found in a book by Donald Kennedy called Academic Duty (Ref 32).

Clinical Trials Using Purified Human HSC to Regenerate the Blood Forming System of Patients Receiving Otherwise Lethal Doses of Chemotherapy

Modern cancer chemotherapy, the attempt to kill cancer cells spread throughout the body in a predictable way, is derived from an understanding of spontaneous mutations in phage and bacteria. In the 1940s Luria and Delbruck (Ref 33) and Lederberg (Ref 34) found that spontaneous variants of genes that, for example, determine antibiotic sensitivity or resistance, exist in populations of microorganisms prior to their exposure to the selecting agent such as the antibiotic. These usually exist in an unselected population at a frequency of about 1 in 10^6 organisms. If one starts with a population of 10^{11} to 10^{12} organisms, it is a virtual certainty that there will be many variants resistant to any single antibiotic or chemotherapeutic agent. The probability that any single bacteria will be resistant to two agents acting on different molecular targets within the cell is the product of that probability, about 10^{-10} to 10^{-12}. The probability of spontaneous resistance to three independent agents is infinitesimal, unless the resistance is derived by multidrug resistant transporters that remove most or all hydrophobic agents from the cell (Ref 35). Modern cancer chemotherapy usually involves simul-
taneous administration of three or four independent chemotherapeutic agents, plus or minus local or whole body radiation. In the 1960s, this led to the first cure of childhood acute lymphocytic leukemia (Ref 36). For many cancers, however, the dose to kill all existing cancer cells in the body is, as described above, at a level which kills hematopoietic stem cells down to the level that regeneration is not possible before death. In theory, these patients could be cured by removing their bone marrow before these myeloablative therapies are instituted, freezing it, and rescuing them after the chemotherapy administration is finished (for review see 37). However, for many late stage cancers and leukemias the spread of metastatic cancer cells also contaminates blood and bone marrow. It makes little sense to go to the trouble to destroy all cancer cells within the body, if you follow that with re-seeding of the body with marrow transplants or mobilized blood transplants containing cancer cells. But we were able to demonstrate that high speed FACS isolation of HSC using two or more marker antibodies resulted in at least a 100,000 fold depletion of contaminating cancer cells (breast cancer cells, or non-Hodgkin’s lymphoma cells, or multiple myeloma cells), whereas flow or magnetic particle or bead isolation for enrichment of HSC using a single monoclonal antibody (anti-CD34) was insufficient to remove all cancer cells (Ref 7, 38). Thus we could for the first time deliver back to the patient HSC with little or no contaminating cancer cells from their own bodies. Three phase 1/2 clinical trials were carried out with prospectively isolated human HSC: stage IV (widely metastatic) breast cancer (Ref 39), non Hodgkin’s lymphoma (Ref 40), and multiple myeloma (Ref 41). In all three trials the purified HSCs were given at doses that enable very rapid return of white cells and platelets, comparable to the time of regeneration of these blood elements within fractionated bone marrow or immobilized blood (Ref 39-41). At about four years post transplantation about 35 to 40% of Stage IV breast cancer treatment patients receiving this treatment were alive without evidence of disease, and about 55% of the non Hodgkin’s lymphoma were alive without evidence of disease (R. Negrin, personal communication and Ref 39). In different trials using unpurified mobilized peripheral blood, the four year Stage IV breast cancer survival without disease progression was about 6% (Ref 42). The data for patients with multiple myeloma are still being collected. Because these were not experiments where patients were prospectively randomized into pure stem cell versus unmanipulated mobilized blood transplant, it is not possible to tell whether these increased cancer free survival rates are significant and reproducible, so further trials are warranted.
Transplantation of HSC from Genetically Distinct Donors Enables Donor Specific Tolerance of Tissue Grafts, and Donor Specific Abrogation of Autoimmune Diabetes in Mice

Transplantation of HSC from marrow or mobilized blood between genetically distinct donors, even if they are matched at the major histocompatibility (H-2) in mice (and HLA in man) (Ref 43, 44), leads to a serious complication; T cells from the donor make an immune attack against all host tissues, called graft vs host disease (GVHD) (for review see 45). Purification of HSC in mouse and man completely eliminates contaminating T cells. We have carried out many HSC transplants in different mouse strain combinations, some matched at the MHC but different otherwise, and others where no match at MHC (H2), or other loci were expected. Higher doses of HSC were required in the allogeneic mismatched transplants than in syngeneic transplants to achieve rapid and sustained engraftment (Ref 46-48). We also showed that hosts whose immune and blood forming systems were generated from genetically distinct donors were permanently and specifically tolerable of donor and host tissue and organ transplants, whether the organ transplants were given the same day as HSC, or up to a year later (Ref 47, 49). We have now achieved submyeloablative regimens for such combined HSC and organ transplants, so that one can expect that the host would not be at risk for death by the regimen that conditions the host for HSC engraftment (Ref 46, 50). Translation to man should enable a switch from chronic immunosuppression for organ, tissue, or other tissue specific stem cell transplants to protocols wherein a single conditioning dose allows HSC and tissue, organ, or other tissue specific stem cell engraftment for life. This should eliminate both GVHD and chronic host transplant immunosuppression, which leads to many complications, including life threatening opportunistic infections and the development of tolerated malignant neoplasms.

These mouse preclinical experiments also allow one to assess whether various genetically defective hematolymphoid systems and hosts can be replaced by healthy blood forming systems. These include not only genetic defects such as aplastic anemia, thalasemia, severe combined immunodeficiency and sickle cell anemia, but also replacement of immune systems prone to attack self that create auto-immune diseases (Ref 6,50). These auto-immune diseases include Type 1 (juvenile) diabetes (autoimmune T cells destroy insulin producing pancreatic islets) (Ref 51-53), multiple sclerosis (autoimmune T cells destroy myelinating oligodendrocytes), rheuma-
toid arthritis (Ref 54), systemic lupus erythematosus, and ankylosing spondylitis, to name a few of the genetically-determined autoimmune disorders. We have shown that normal HSC can replace the autoimmune-prone hematolymphoid system of mice (NOD) with developing Type 1 diabetes at the stages in which polydypsia and polyuria precede complete islet destruction; these mice are cured of the development of this disease (Ref 50). Mice already having complete destruction of the islets can also have the autoimmune part of their disease abrogated with HSC transplants from donors lacking the genetic risk for diabetes, and in some cases frank diabetic animals treated with HSC and islet transplants from healthy donors are also cured of their need for insulin and the complications of their disease (Ref 50). Many organ systems are susceptible to these kinds of autoimmune attacks, and so we need to search for engrafting or stem cells specific for each of these tissues and organ systems, such as pancreatic islets, myelinating oligodendrocytes, cartilage producing chondrocytes, and liver cells, to name a few. The subject of other stem cell types will be addressed later in this manuscript.

Expansion of HSC

The number of HSC that one can isolate from mobilized blood, or from umbilical cord, or from bone marrow limits the full application of HSC transplantation in man, whether in response to accidental or intentional nuclear radiation exposure, or transplantation in the treatment of diseases as described above. Engraftment times of 50 days or more used to be standard when limiting numbers of bone marrow or umbilical cord bloods were used in a transplant setting, reflecting the low level of HSCs found in these native tissues. Attempts to expand HSC with the known cytokines stem cell factor/steel factor (SLF), thrombopoietin (TPO), interleukins 1,3,6,11, plus or minus the myeloerythroid cytokines GMCSF, GCSF, MCSF, and erythropoietin have never resulted in a significant expansion of HSC. Rather, they induce many, if not most HSCs into cell divisions which are accompanied always by cell differentiations (Ref 55). Yet there are many experiments wherein single or a few HSC were transplanted into animals, and in those settings animals expanded the number of HSCs at least 100,000 fold at the steady state while permitting the daughters of HSCs to regenerate full blood forming systems (Ref 21, 22, 23, 24). Thus we did not have in hand the factors that were present in the body to regenerate HSCs by self-renewing cell divisions. We have recently found the pathway that
enables at least mouse HSC to undergo massive self-renewing cell divisions, with progeny that are functional in hematopoietic cell transplantations into lethally irradiated hosts (Ref 56, 57). By investigating genes transcribed in purified mouse LT-HSC we have found that these cells contain expressed elements of the Wnt/fzd/beta-catenin signaling pathway (unpublished data of E. Ranheim, S. Prohaska, C. Forsberg, A. Wagers, K. Li, S. Cheshier, and I. Weissman). Wnt was first discovered as a mouse gene rescued by milk transferred (Ref 58) mammary tumor virus (Ref 59) upon its integration into breast cells, resulting in uncontrolled proliferation of breast cells resulting in breast cancer (Ref 59, 60). Transgenic mice having mammary cell specific enforced expression of that Wnt developed breast cancers (Ref 60). It was shown in Drosophila that Wnt was a secreted ligand for a frizzled (FZD) receptor, and specified developmental commitments (for review see Ref 61). The interaction between Wnt fzd and the complementary FZD receptor along with a coreceptor related to LDL receptors (LRP6) resulted in the release of cytoplasmic beta-catenin from a multiprotein complex that phosphorylated and destroyed beta-catenin (Ref 61). Released, unphosphorylated beta-catenin, translocated to the nucleus, where it bound to DNA binding proteins of the LEF/TCF family, converting them from repressors of transcription to activators of transcription (Ref 61). Proteins in the multiprotein destruction complex are negative regulators of beta-catenin activation and stimulation of LEF/TCF transcription, and it has been found empirically that destructive mutations of each of those negative regulatory elements can play a role in the development of at least colorectal carcinomas (Ref 62). Breast cancers and colorectal cancers are diseases wherein uncontrolled self renewal leads to expansion of malignant cell populations that we call cancer stem cells ([63] see below). We then tested the possibility that the Wnt/Fzd/beta-catenin pathway is a regulator of self-renewing divisions of at least mouse HSC, and demonstrated that addition of highly purified Wnt3A to HSC leads to their expansion, as does transfection of activated beta-catenin genes into HSC (Ref 56, 57). HSC cell lines requiring no serum could be established that expanded 100 to 1000 fold, and these expanded HSC were also transplantable (Ref 56); such tissue culture expansions of HSC in serum-free conditions could only be accomplished if these cells were blocked in the programmed cell death pathway by BCL-2 (Ref 55, 64, 65). Wnt activation of HSC leads to up-regulation of other genes implicated in HSC self renewal, including Notch 1 and HOX B4 (Ref 56, 66, 67). Therefore it is critical to discover whether the same pathways operate in the expansion of human HSC, and if we can take
advantage of these pathways to expand rare populations of HSC at will. In that way HSC transplants might be possible starting from small collections of HSC rather than massive mobilizations and apheresis (see below) and one might convert collections of HSC from volunteer donors or umbilical cords into storable expanded and aliquotted stem cell banks useful on demand for clinical transplantation and/or for protection against radiation accidents. In mice successful HSC transplants that regenerate fully normal immune and blood forming systems can be accomplished when there is only a partial H-2 (mouse MHC) match, and the establishment of useful human HSC banks might require as little as a 3 out of 6 match of subcomponents of the HLA gene haplotype. This might be accomplished with stem cell banks of as little as 4-10,000 independent samples.

HSCs Normally Traffic From Bone Marrow to Blood, and This Can Be Greatly Amplified by HSC Mobilization Protocols

Research in the late 1950s and early 1960s indicated that bone marrow in mice was the major source of hematopoietic reconstituting cells, the mouse spleen was about one-tenth as efficient and mouse blood about 1/100th as efficient (Ref 68). But we did not know whether the blood HSC had recently derived from marrow, or if they represented a stable recirculating population. The optimal mobilization regimen for HSC currently used in the clinic is to treat the marrow donor with a drug such as cytoxan which kills most dividing cells (cytoxan is a nitrogen mustard derivate). Normally, only about 8% of LT-HSC enter cell cycle per day (69), so these are hardly affected by a short treatment with cytoxan, but most of the downstream multipotent and oligopotent progenitors are mainly in cell cycle (70, 71) and their numbers are greatly depleted by this dose, creating a demand for hematopoiesis to regenerate a blood forming system. Empirically, cytokines such as GCSF and SLF can increase the number of HSC in the blood, especially if administered for several days following a cytoxan pulse. We have shown that the optimized protocol of cytoxan plus G-CSF results in every resident LT-HSC in mouse bone marrow undergoing several self-renewing cell divisions, expanding the number of HSC 12 to 15 fold in a matter of two to three days (Ref 72, 73). It appears that on the second or third day following mobilization, up to one-half of the daughter cells of self-renewing dividing LT-HSC leave the bone marrow, enter the blood, and within minutes engraft other bone marrow, spleen, or even liver hematopoietic sites (Ref 74). In normal mice transfused LT-HSC also rap-
idly emigrate from blood to hematopoietic tissues (Ref 74), and to maintain the steady state of 100 LT-HSC the bone marrow must produce and release between $10^4$ and $10^5$ LT-HSC per mouse per day into the blood stream (Ref 74). Resident cells in the blood undergoing this massive HSC flux can and do enter empty hematopoietic niches elsewhere in the bone marrow, and provide sustained hematopoietic stem cell self-renewal and hematopoiesis (Ref 74). We assume that this property of mobilization of HSC is highly conserved in evolution (it has been shown in mouse, dog and humans), and presumably results from contact with natural cytotoxic agents in the wild, after which regeneration of hematopoiesis requires restoring empty HSC niches. This means that coursing through each and every tissue of the body in very large numbers everyday in normal individuals are functional, transplantable HSC. Concurrent with the discovery of this massive HSC flux through all tissues were the early claims that brain cells in developed animals contained stem cells that could turn into blood forming stem cells (Ref 75), that bone marrow blood forming cells could give rise to neurons in the brain (Ref 76, 77), skeletal myocytes in muscle (Ref 78-80), regenerating myocardium and blood vessels in the heart (Ref 81-83). These latter studies were not carried out in a way, initially, that would distinguish between tissue specific stem cells coexisting with itinerant HSCs in the tissues; but these were in fact experiments wherein it was claimed that a single stem cell population within the tissue could give rise to resident tissues (myocardium, skeletal muscle, brain) as well as blood formation (Ref 84). These experiments were the basis of claims that demonstrated the plasticity of adult stem cells, and were contrasted to the embryonic and fetal development of organs and tissues, wherein commitment appeared to be from more pluripotent to multipotent to oligopotent to unipotent cells, without transdifferentiation of one germ line tissue (for example mesoderm) to another (for example ectoderm or endoderm). We discuss below where these well publicized claims stand currently.

Can Blood Forming Stem Cells or Other Resident Bone Marrow Cells Transdifferentiate to Other Tissue Specific Stem and Progenitor Cells, or Can They Contribute to Regeneration of Nonhematopoietic Damaged Tissues?

We had called into question the claims that one adult tissue type stem cell could turn into another tissue type cell (Ref 22, 85, 86). We proposed that one could only claim one tissue could turn into another if one began with highly purified well known tissue committed stem cells, best were sin-
gle stem cells (for example, HSC), transferred to genetically distinct hosts, and demonstrated the progeny of that stem cell included both its normal tissue derivatives as well as other kinds of tissues (blood and other tissues in the HSC case). The existence of large numbers of circulating hematopoietic stem cells through each tissue requires this kind of single cell analysis, or purification to homogeneity of cells. The initial claim that skeletal muscle contained a common stem cell for blood and muscle (Ref 83), was later amended when this second criterion was applied, and it turned out that the cells giving rise to the blood and blood forming system were simply hematopoietic stem cells in muscle with hematopoietic stem cell markers, whereas the population giving rise to muscle were cells within muscle lacking hematopoietic lineage markers like CD45, and that could give rise to muscle only; it is not yet clear whether these are purified muscle stem cells (Ref 87). There was a second claim that neurospheres derived from a clonogenic brain cell could give rise in vitro to brain cells, but upon injection in vivo, and after a delay of about seven months, could replace the full hematopoietic system of sublethally irradiated allogeneic hosts (Ref 75). This failed replication. There was another claim that single surface model mouse brain cells with 80% neurosphere-initiating cell potential could be isolated, and these cells could give rise to at least muscle and brain (Ref 89). We cannot replicate that finding (Raveh, T., Pham, K. and Weissman, I.L., in preparation). There is another experiment wherein bone marrow cells depleted of mature and maturing lineage positive cells, labeled in vitro with a vital dye, could be transplanted into an irradiated host, and cells not undergoing cell division reisolated from bone marrow two days later; these cells at the single cell level were reported to reconstitute the blood forming system and many epithelial tissues in irradiated mice (Ref 84). None of these experiments have been repeated in the published literature.

In another experiment single, visually-observed lateral ventrical ependymal cells were isolated, cultured extensively to produce neurospheres, and the daughter cells placed in the blastocyst of the same species (mouse) or directly injected into a developing chick embryo; donor markers could be found in a wide variety of tissues, though oddly excluding hematopoietic tissue (Ref 90), different than tissue produced from another neurosphere experiment (Ref 75). One published attempt to reproduce this experiment failed (Ref 91). There are several experiments wherein bone marrow cells, often characterized as hematopoietic stem cells but without the attendant purification, injected into lethally irradiated animals give rise to donor derived myofibers in muscle and cortical neurons as well as cerebellar
Purkinje cells in the brain (Ref 76-82). We and others have attempted to reproduce the demonstration of production of cortical neurons from marrow or hematopoietic stem cells precursors, and have failed (Mei, H., Wagers, A. and Weissman, I.L., unpublished data). However, donor markers can appear in muscle, in Purkinje cells in the cerebellum, rarely in myocardial epithelium, and in liver cells (Ref 81, 92, 93 and see below). There are experiments wherein bone marrow cells and cells enriched but not highly purified for hematopoietic stem cells injected into the injured myocardium shortly after a left anterior descending coronary ligation has been carried out, and the donor cells are claimed not only to give rise to cardiac muscle, blood vessel, and perivascular smooth muscle cells containing donor markers, as well as providing a functional regeneration of the heart (Ref 81, 100). These experiments have led to extensive clinical trials in humans (Ref 95-99). We have carried out extensive experiments attempting to repeat these findings, and always find that either hematopoietic stem cells or bone marrow cells having donor markers can be found in the inflamed and damaged cardiac muscle, but none of those cells express the markers, the morphology, nor the function of cardiac muscle, or of cardiac blood vessels, or of cardiac smooth muscle cells (Ref 101).

Cell Fusion and Claims of Stem Cell Plasticity

The fusion of two cells to produce a heterokaryon, a cell with two different nuclei, is a rare event in nature and in pathology, but it has been recorded at several different levels. First, in many pathological states following infection with agents as widespread as Sendai virus or Mycobacterium tuberculosis, multinucleated giant cells derived from the fusion of cells of the monocyte/macrophages series are a constant and often pathognomonic sign of the infection type (Ref 102). Second, cells of the monocyte/macrophage series normally fuse by the thousands every day in every bone to form osteoclasts, cells whose phagocytosis and digestion processes remodel the bones to make space for bone marrow (Ref 103). Third, the Sendai virus from Sendai virus pneumonia has been used experimentally to produce artificial heterokaryons since the mid 1960s (Ref 104-106), and these have been used to understand the role of nucleus and cytoplasm in determining gene expression profiles (Ref 106). Fourthly, Sendai virus and polyethylene glycol (PEG) have been used experimentally since the mid 1970s to create heterokaryons between normal antibody forming cell precursors and malignant plasma cell line cul-
tures in order to make hybridomas that produce monoclonal antibodies (Ref 18, 107). Fifth, in the normal development of all skeletal muscles, myoblast progenitors fuse to each other or to existing myotubes to form multinucleated skeletal muscle cells (Ref 108), a physiological phenomenon. Sixth, true embryonic stem cells derived from mouse inner cell mouse blastocysts have been shown when mixed together in vitro to fuse as well (Ref 109, 110). It is unclear which of these cell fusions is physiological and which pathological, especially when cells of the monocyte macrophages lineage are involved, whose normal function is to adhere to dying and dangerous cells during the process of phagocytosis. Helen Blau reviews elsewhere in this volume her argument that cell fusions represents a physiological repair and rejuvenation process (111, 112). As described above, there are several well documented cases wherein stem cells or tissues containing stem cells, can upon in vivo injection give rise to the donor markers in other tissue types cells. In the first case, for example, purified hematopoietic stem cells could contribute donor markers as well as a functional enzyme to highly damaged liver cells in a host lacking FAH (Ref 92). In these experiments the donor marker positive cells underwent several rounds of drastic selections; FAH lacking cells die from toxic products such as fumary/acetol acetic acid, and only those cells that contained normal FAH are selected and can survive. In fact significant and functional liver regeneration in those situations occurs only with a few animals and only after months of repeated selection, as most hosts died during the selection. A systematic analysis of the chromosomes in these regenerated livers have demonstrated that in every case there was a fusion between host and donor hematopoietic cell progeny (Ref 93-94). Mice restored with highly purified hematopoietic stem cells, some with a single hematopoietic stem cell that gives rise to hematopoiesis, or reconstitute with whole bone marrow containing that number of stem cells used in the hematopoietic stem cell reconstitution were tested with and without cardiotoxin and crush damage to skeletal muscles in a variety of muscles to test for donor markers in skeletal muscle (Ref 113). For most muscles bone marrow give rise to donor marked myocytes in a cell fusion with an efficiency far better than HSC transplanted hosts, even though the bone marrow that was transplanted in these instances have the same number of HSCs as the host (Ref 113). In no cases did this exceed 0.1% of myofibers and all were cell fusions. Thus rare cell fusions in for the most part highly damaged muscles do come from blood borne circulating precursors (Ref 113). In addition to the two well described cases of liver
and muscle, donor derived nuclei have been found to reside in the same cell body as a host nucleus in bone marrow transplanted animals in the case of the rare Purkinje cells in the cerebellum (Ref 22, 94, 114), rare cells in cardiac muscles that have donor markers (Ref 94), and rare liver cells (Ref 93, 94). The brains of mice restored with purified hematopoietic stem cells and or bone marrow do contain a class of normal hematopoietic cell derived microglia, and these can increase in frequency over time; many of these microglia have long processes, but retain markers of the myelomonocyte lineage (Ref 115). There are several recent accounts that bone marrow derived cells can give rise to regenerating pancreatic islet beta cells producing insulin (116, 117). Finally, mesenchymal stem cells (MSC) isolated by a particular protocol have been reported to be fully pluripotent (119, 120). These claims have been presented to and published by the President's Bioethics Council.

The claims in the above paragraphs received broad public attention before the demonstrations that they might not be reproducible, usually even before the manuscripts were published in peer reviewed journals. These claims of transdifferentiation plasticity of adult tissue stem cells appeared to contradict extensive studies on the embryological origin of the three tissue germ lines, and the tissues derived from them (Ref 118) and were taken in public venues to mean that adult stem cells were also pluripotent, and obviated the need for the study of pluripotent stem cells derived from the blastocyst stage of development, whether this blastocyst derived from a sperm-egg fusion or nuclear transfer (see below). Several self-described stem cell experts and the organization Do No Harm, cited 228 publications claiming adult stem cell activities and transdifferentiation in a letter to Ruth Kirschstein, then Director of NIH. However, on close inspection zero out of the 228 papers fit the criteria described in Refs 85, 86 that a known stem cell of one type (perhaps at the single cell level), in an in vivo differentiation system demonstrated the functional regeneration of cells from another tissue (I. Weissman, personal observations). Some members of the US Senate, US House, and the President's Bioethics Counsel (121, 122) also cited such claims in developing their own positions against the use of human pluripotent stem cells derived from the blastocyst stage of development as a legitimate object of scientific and medical study in the United States. Before I consider the scientific explanations for those few experiments wherein donor markers were found in different types of host tissues I should reiterate what is generally accepted in the scientific community as a discovery and an established finding.
Without the triad of published accomplishments, shown in Table 1, it is inappropriate for any body or agency to take the initial claims as true enough to affect clinical care protocols or public funding and policy decisions.

Using the criteria in Table 1 for the acceptance of an experimental phenomenon as a biological fact, we do not believe there is sufficient evidence for any of the transplant claims of transdifferentiation. In fact, most if not all reports of donor markers in unexpected tissues are the result of cell fusions, and the rarity of cell fusions makes it questionable that such events are regenerative rather than reflect the functions of post-injury phagocytic cells.

It is conceivable that some of these very rare cell fusions could be part of a regenerative process, but there is no evidence today that such is the case. Such evidence would require that donor marked cells fusing with generating or regenerating host cells contributed to a robust regeneration process.

Do Other Tissues Have Tissue Specific Stem Cells Used in Their Generation and Regeneration Throughout Life?

In addition to hematopoietic stem cells, the following stem cells have been prospectively isolated to homogeneity: peripheral nervous system stem cells (Ref 123, 124) and central nervous system stem cells (Ref 125-127). In addition highly enriched populations that contain stem cells have been found for the skin (Ref 128), as well as mesenchymal stem cells (Ref 119, 129, 130, 131, 132). In the case of human CNS stem cells, extensive experiments using their transplantation into the lateral ventricles or into the brain or into the spinal cord of SCID mice have shown that they con-

---

**TABLE 1**

The Transition from Discovery to Accepted Scientific Fact

1) The initial discovery must be published in fully peer-reviewed journals.
2) The experiment as published must be repeatable in many independent laboratories.
3) The phenomenon described should be so robust that other experimental methods must reveal it.
tributed in a robust way to engraftment of the neurogenerative cells (the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus in the hippocampus) in adult animals, as well as daughter cells that appear to be undergoing site specific self-renewal, migration, and differentiation into oligodendrocytes, various neurons, and astrocytes in an apparent site-appropriate manner (Ref 127, 133). These cells can remyelinate mouse axons in the Shiverer myelin deficient mice, can participate in the formation of appropriately placed interneurons in the olfactory bulb and elsewhere, can participate to ameliorate the effects of fresh crush-type spinal cord injuries (Ref 134, 135), and can migrate selectively towards an ischemic area in the central nervous system following middle cerebral artery blockage in the immunodeficient mouse (Ref 127, 133-136). They can also provide PPTI enzymes to PPTI deficient mice with Batten’s disease, ameliorating the build-up of lipoprotein lipofuscin deposits and the loss of neuronal cells and their axonal and dendritic processes (Ref 137). These robust regenerations stand in contrast to microglial contributions and the rare Purkinje cell fusion derived from bone marrow and hematopoietic stem cells (Ref 138-139).

We do not yet know that all organ systems are based on the stem → progenitor → progeny model, but the method that led to the isolation of hematopoietic stem cells, peripheral nervous system stem cells, and central nervous stem cells is general enough to look for such tissue and organ specific stem cells. It should be added that the same method was used for prospective isolation of most if not all hematopoietic progenitor cells downstream of hematopoietic stem cells in mouse and man, and these cells too are capable of more limited and more specific regeneration of various important blood cell types, although none of these cells self-renew, and therefore all such transplants are transient in nature (Ref 140-152).

Cancer Stem Cells

Elsewhere we and others have provided evidence that cancerous tissues, which are obviously derived from normal tissues following somatic genetic and epigenetic changes, contain within them cancer or leukemia stem cells, and for the most part these cancer and leukemia stem cells differ from normal tissue stem cells (153-163) as well as the nonmalignant progeny they produce in a failed attempt to make normal tissue. These can include progenitor cells within tissues that have acquired the capacity for self renewal, as well as multiple genetic and epigenetic events to avoid pro-
grammed cell death, immune mediated death and phagocytosis, and limitation of replication life spans by loss of telomere protective sequences (Ref 164-171). The subject of cancer and leukemia stem cells is better described elsewhere (Ref 159).

Nuclear Transplantation to Produce Reproductive Clones and Nuclear Transplantation to Pluripotent Stem Cell Lines

A central tenet of developmental biology is that following sperm-egg fusion the early preimplantation embryo develops two kinds of specified cells – the pluripotent cells within the inner cell mass, and the trophoblasts/placenta committed cells that surround the inner cell mass of the preimplantation blastocyst (Ref 172). Key experiments on transfer of cells just after implantation from the implanted mouse conceptus to genetically distinct preimplantation mouse blastocysts reveals that pluripotentiality is lost as the cells follow epigenetic programs to commit to maturing and different cell fates, first the formation of the three germ layers, and later the tissues derived from them (Ref 172, 173). Except in the case of mature lymphocytes, there is no evidence that the nuclei of highly differentiated cells have lost genetic material, and in the case of lymphocytes, it is merely the potential to rearrange subgenic elements into new antigen receptors and the high degree of variability they endow to an immune system that must have diversity to protecting against infections and neoplasms (reviewed in 174, 175). Therefore it was quite surprising when, in the 1950s and 1960s, Briggs and King; and Gurdon provided evidence that the frog has cells after the blastocyst stage which upon transfer into the enucleated egg can participate in early (and rarely later) developmental events, implying that the epigenetic events that change the differentiation commitments and functions of various cells might be, at least in part, reprogrammed to that of pluripotentiality (Ref 176, 177). However, this field lay fallow for many years thereafter, and reemerged in a popular and in a scientific sense when Ian Wilmut reported the birth of a cloned sheep (Ref 178). Dolly was derived by nuclear transfer of a cell found in the mammary gland into an enucleated sheep egg which was carried in vitro to the blastocyst stage before implantation in a prepared sheep uterus (Ref 178). It was later shown in mice that the same kind of nuclear transfer technology could give rise, rarely, to preimplantation blastocysts, and rarely, from these, pluripotent stem cell lines (179-180) could be derived that were similar in properties to the embry-
onic stem cell lines that had previously been isolated from the inner cell mass of mouse blastocysts (Ref 181, 182). At about the same time as the publication of the sheep reproductive clone called Dolly, Thomson et al. in Wisconsin reported adaptation of the technology first used to produce mouse embryonic cell lines from mouse blastocysts (181, 182) to the production of human embryonic stem cell lines from human preimplantation blastocysts (available from in vitro fertilization clinics) (Ref 183). Hogan and Donovan (Refs 184, 185) had shown that the primitive germ cells that exist in the mouse embryo between 9 and 11 days of gestation prior to their arrival in the genital ridges and commitment to gamete outcomes, could be used to produce mouse pluripotent stem cell lines. Gearhart et al. used culture conditions, adapted from the mouse studies, to produce human pluripotent stem cells lines from human primitive germ cells (Ref 186).

Briggs and King (176), Gurdon (177), and over 30 years later, Wilmut et al. (Ref 178) demonstrated that nuclear transfer in several subprimate species could result in sufficient reprogramming of the nucleus to allow early stages of development to be revealed, and rarely developed organisms to be born (by reproductive cloning). This opened the possibility that one might be able to produce human pluripotent stem cells lines not just from those blastocysts resident in various in vitro fertilization clinics but also from predefined genotype donors; such pluripotent stem cells developed in mice by nuclear transfer (NT) lines have the genetic characteristics of the cell nucleus donors (179, 187).

The possibility of human reproductive cloning and the possibility of NT to produce human pluripotent stem cell lines led the Presidents of the National Academies to assemble a panel of scientists, physicians, and medical ethicists to examine in an unbiased manner the scientific, medical, and medical ethics issues surrounding nuclear transfer technologies both for reproductive cloning and to produce human pluripotent stem cell lines, and to report back to them with recommendations. I was chairman of that panel (Ref 188). The panel had at its disposal the ability to research all areas relevant to the subject, to hold a workshop (which was on August 7th, 2001) to make sure that the panel had current knowledge of all attempts in animals at reproductive cloning and production of pluripotent stem cell lines by nuclear transfer. This workshop was arranged as an open forum so that all would-be reproductive cloners could state the medical and scientific basis of their plans, hear the actual outcomes of animal research in the area, and question and be questioned
We found that in over 17,500 attempts at reproductive cloning in at least five mammalian species, about 99.2% of those implanted blastocysts died in utero. Of those that were born, many died soon thereafter (Ref 188). In the case of many species a common syndrome was discovered to be dangerous to the life of the fetus and the mother that bore it – the large offspring syndrome; this syndrome is due to defective placentation (Ref 188). Given these and other examples, and given the lack of evidence that any would-be cloners or any animal reproductive cloners had developed technologies that in the future would advance or change these outcomes, and taking into account a long history of the medical ethics of human participants in medical research, starting from the Nuremberg Code (Ref 189), the panel voted unanimously to call for a legally enforceable ban on human reproductive cloning. The panel defined reproductive cloning as implantation of blastocyst stage nuclear transfer products into the uterus with the intent of reproductive cloning (Box 2 from Ref 188).

We also examined the issues surrounding NT to produce human pluripotent stem cells lines. There are at least four areas of research that could not be accommodated with already established human embryonic stem cell lines, all derived from in vitro fertilization clinics (Ref 190). The first was to diversify the genetic diversity of the human ES cell lines. Although it was claimed that 64 such cell lines existed for experiments funded by the US government in President Bush’s August 9, 2001 executive order, in fact, very few of those cell lines proved to be available for wide use. More importantly, they only represented the ethnic and racial diversity of people who need assisted reproductive technologies to establish a pregnancy, and in the United States this includes a bias for people who are Caucasian, who are well-to-do, and always includes people who are infertile, a frequent abnormality that may have a genetic component.

A second reason is the possibility of ‘therapeutic cloning’ wherein the individual who donates the nucleus to produce a pluripotent stem cell line is recipient of cell products from that cell line. Because the nucleus of any body cell contains genes encoding most of the major and minor histocompatibility antigens (mHC and MHC respectively), it was hoped that stem and progeny cells from a donor derived pluripotent stem cell line would be histocompatible with that donor. This is true for the nucleus and chromosomal encoded genes, but there is extensive genetic polymorphism of mitochondrial genes and it was well established in studies of mice that the proteins produced by the mitochondrial genes can serve
as peptides for MHC presentation, which are mHC (Ref 191, 192). In nuclear transfer to produce pluripotent stem cells, the egg mitochondria usually are retained and therefore give rise to mitochondria in the pluripotent stem cell lines produced from them. Nevertheless such immune responses to mitochondrial specified mHC are relatively easily overcome with low doses of immunosuppressive drugs so this is not an ultimate barrier to the eventual practice of therapeutic cloning. In fact, there is an excellent mouse example of therapeutic cloning, wherein the somatic cell nucleus from a genetically immunodeficient mouse was used to create a pluripotent stem cell line, and the genetic defect that had led to the immunodeficiency was corrected in the cell line (Ref 187, 193). These gene-corrected ES lines were converted to transplantable hematopoietic cells in vitro, and those cells were transplanted into the genetically immunodeficient mice, partially curing their immunodeficiency (Ref 193). Therapeutic cloning represents the possibility that one can find stem and progenitor cells for tissues that have not yet yielded their adult phase stem cells for transplantation, as well as the possibility that organs or tissues produced this way can provide life saving transplants when the patients own organs or tissues are irreversibly damaged.

A third, and I believe the most important reason for doing nuclear transfer to produce human pluripotent stem cell lines rests with the finding in mice that genetic abnormalities contained in the donor somatic cells, in the case of the immunodeficient mouse, skin fibroblasts, give rise to cell lines that faithfully reproduce the genetic abnormality in the pluripotent stem cell line, and in mature tissues derived from the pluripotent cell line (Ref 187). An example of a genetic immunodeficiency was described in the previous paragraph. Other mouse pluripotent stem cell lines derived by nuclear transfer from T lymphocytes or B lymphocytes had the specified rearranged immune receptor genes of the nucleus donors (Ref 179). Thus, every time that a known genome from a donor cell was used to produce a pluripotent stem cell line, the genetic program of that genome was replicated in the cell line derived from it and in mice, the in vivo tissues and organs.

The human genome project has provided for us the tools to discover the many gene alterations that are inherited in families and that cluster in patients with particular diseases. It turns out that a very large number of human diseases are genetically determined or strongly genetically influenced, including diseases as common as type I and type II diabetes, early onset cardiovascular diseases, autoimmune diseases, most neurodegenerative diseases such as ALS, Alzheimer's, Huntington's, etc, many endocrin-
logical disorders, all lysosomal storage diseases, and most cancers, to name a short list of the large number that are present. To reiterate, the human genome project is changing our understanding of the inheritance of these diseases, from knowing which chromosomes carry the familial traits to identifying the genes that show correlated genetic differences with the diseases. But understanding which genes are involved in a particular disease does not allow one to understand the pathogenesis of these diseases. For virtually all of these genetically determined or genetically influenced diseases, the role that each of the particular mutant genes (that correlate with the disease) plays in the pathogenesis of each disease is simply unknown. For example, not everybody who has the high risk MHC gene for type I diabetes actually gets diabetes; several other unlinked gene loci are involved. The person with the disease had an unfortunate inheritance of the high risk genes to develop this disease. This is also true of amyotrophic lateral sclerosis, (Lou Gehrig's Disease), and in fact most of the diseases cited above. Understanding the pathogenesis of the disease requires being able to take its components apart in a reductionist manner, and the many steps in that reductionist exercise that are relevant cannot be done with living patients, or even tissue from patients taken post mortem. For these multigenic diseases that involve more than one tissue or organ system, it is a very important and daunting task to be able to put together how development or function goes awry in any particular disease. But if one had a pluripotent stem cell line, or several, from patients harboring known genomes and known genetically determined diseases, and could compare them to people in the same families who don’t have the disease, one can begin to identify the pathogenic genes; and by isolating the adult-type stem cells that are involved in their development, as well as transplanting these maturing cells into the cognate organ of severe combined immunodeficient mice, one can follow for the life of the mouse the potential pathogenesis of the disease in each of the interacting organ systems (Ref 127, 194-196). One could therefore use such genetically determined disease-derived pluripotent stem cell lines to carry out reductionist experiments in vitro and in vivo to begin to determine which of the correlated gene abnormalities are more important in the causation of the disease than others, in which order, and in which tissues they occur; and by gene correction techniques, show whether the particular disease susceptibility was ameliorated, validating that gene and it’s product as a target for therapeutic intervention.

The fourth kind of pluripotent stem cell research in humans that cannot be carried out with existing human embryonic stem cell lines is the use
of nuclear transfer technology of cells that have diverged from their inherited genes by a process of somatic mutations and/or alterations of gene expression. These include all cancers and leukemias, and some neurodegenerative diseases such as Huntington’s disease. There is no reason to believe that the genetic changes that lead to the generation of cancer stem cells and leukemia stem cells alter the ability of the genome of those cells to undergo reprogramming following nuclear transfer to establish pluripotent stem cell lines. Therefore, in addition to inherited genetic diseases, NT pluripotent stem cell lines from all patients with cancers can fall under the same reductionist approaches. This would require the identification and isolation of cancer stem cells for each different type of cancer, production of human pluripotent stem cell lines from them by nuclear transfer, and identification by genome analysis of genes mutated or changed in their gene expression profiles in the cancer stem cells.

From the above it is obvious that we are talking about hundreds of diseases that afflict a very significant proportion of humans, and that knowledge of which genes are important for which particular diseases could lead to innovative approaches of these validated targets of experimental therapeutic inquiry, whether it be by pharmaceutical corporations, or for gene therapy, or even for cell therapy groups within and outside academia. For all of these constituencies it is urgent to be able to wrestle with the technological barriers that stand in the way of making nuclear transfer to produce human pluripotent stem cell lines from pre-defined disease donors for the broadest of our medical research communities. Not to do so, or to delay doing so, quite clearly will affect the lives of people who might have been helped when they had a small window of opportunity for relevant therapies derived from the knowledge carried by these cell lines. This changes the equation, from comparing the ethical and religious status of cells in preimplantation blastocysts produced by nuclear transfer to the alternative ethical consideration of the lives of millions of people who have these diseases now, people who are already born (Ref 190). This is a platform technology, much like 25 years ago recombinant DNA was a platform technology. Like recombinant DNA none of us can predict what kinds of discoveries will result if the best and brightest investigators can use these cells in biomedical research institutions, under strict regulatory guidelines. Recombinant DNA technology was regulated, not banned, and hundreds of thousands of patients per year are successfully treated with its products. My personal opinion is that any group that has authority to ban this NT research is responsible for the fate
of patients that could have been treated with relevant therapies created during their short window of opportunity.

These are just a few of the obvious avenues of biomedical research that would be blocked by a ban on research leading to the nuclear transfer to produce human pluripotent stem cell lines from predefined donor nuclei. None of these kinds of experiments can be done with current human pluripotent stem cell lines.

Some Lessons from History

Approximately 75 years ago geneticists were divided as to how natural selection works. One line of thought came from the teachings of Lamarck who held that changes in the selection environment would lead to adaptive changes in the genetics of all organisms, so that over time, one could rapidly develop resistant populations that are heritably altered. This Lamarckian view (in the 1920s) was championed by a Russian geneticist, Trofim Lysenko (reviewed in Ref 200). The alternative Darwinian view stated that rare variants pre-exist in any population prior to the exposure to the selecting environment, so that perhaps one in 100,000 to one in a million organisms already had altered the correct genes for resisting, for example, hostile environments. If one used Darwinian selection methods to isolate resistant organisms to obtain a resistant population, it would obviously take very many generations of selection. When Lysenko became an adviser to Stalin, Stalin chose only to support the Lamarkian approach, and the Darwinian approach was essentially banned (Ref 197-200). Several leading Russian geneticists ended up in jail, and at least one, Theodoseus Dobzhansky, emigrated to the United States (Ref 197-200). Dobzhansky joined with the eminent American geneticist Thomas Hunt Morgan at Cal Tech and together they laid the foundations for modern genetics research, modern biology, and modern medicine, including the recombinant DNA revolution described above. For at least the 50 years that followed this decision, Russia and the Soviet Union produced few or no eminent scientists in the genetics field, the genetic revolution did not occur in the Soviet Union, and the medical treatments that derive from it, as well as the biotechnology companies that develop them, did not happen in the Soviet Union. Thus when governments or societies or religious organizations ban research on the basis of anything but strict scientific and medical merit, medical ethics, and laws made in secular societies, they risk large scale changes for their entire society, and for several human generations.
REFERENCES


20. Uchida, N., Weissman, I.L., 'Searching for hematopoietic stem cells: evidence that Thy-1.1lo Lin- Sca-1+ cells are the only stem cells in C57BL/Ka-Thy-1.1 bone marrow', *J. Exp. Med.*, 175, pp. 175-84 (1992).


33. Luria, S.E., Delbruck, M., 'Mutations of bacteria from virus sensitivity to virus resistance', Genetics, 28, pp. 491-511 (1943).


44. Main, J.M., Prehn, R.T., 'Successful skin homografts after the administration of high dosage X radiation and homologous bone marrow', *Journal of the National Cancer Institute*, 15, pp. 1023-9 (1955).


55. Domen J, Weissman, I.L., 'Hematopoietic stem cells need two signals to prevent apoptosis; BCL-2 can provide one of these, KitL/c-Kit signaling the other', J. Exp. Med., 192, pp. 1707-1718 (2000).


96. Strauer, B.E., Brehm, M., Zeus, T., Kostering, M., Hernandez, A., Sorg, R.V., Kogler, G., Wernet, P., 'Repair of infarcted myocardium by autol-


111. See Blau, H. (See this volume).


113. Sherwood, R.S., Christensen, J.L., Weissman, I.L., Wagers, A.J., 'Sparse contribution to skeletal muscle from circulating cells, bone marrow cells, and hematopoietic stem cells' (Submitted).


128. Taylor, G., Lehrer, M.S., Jensen, P.J., Sun, T.T., Lavker, R.M., 'Involvement of follicular stem cells in forming not only the follicle but also the epidermis', *Cell*, 102, pp. 451-61 (2000).
134. Uchida, N. (Personal communication).


